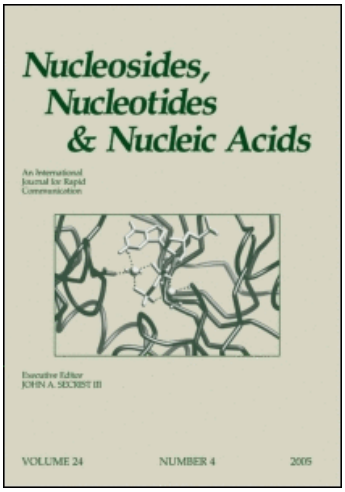


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A Novel and Economical Synthesis of 2''-O-Alkyl-Uridines

Bruce S. Ross[a]; Robert H. Springer[a]; Zeb Tortorici[a]; Stuart Dimock[a]
[a] Isis Pharmaceuticals, Carlsbad, CA

To cite this Article Ross, Bruce S. , Springer, Robert H. , Tortorici, Zeb and Dimock, Stuart(1997) 'A Novel and Economical Synthesis of 2''-O-Alkyl-Uridines', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1641 — 1643
To link to this Article: DOI: 10.1080/07328319708006245
URL: http://dx.doi.org/10.1080/07328319708006245

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1641-1643 (1997)

A NOVEL AND ECONOMICAL SYNTHESIS OF 2'-O-ALKYL-URIDINES

Bruce S. Ross*, Robert H. Springer, Zeb Tortorici and Stuart Dimock
Isis Pharmaceuticals, 2292 Faraday Avenue, Carlsbad, CA 92008

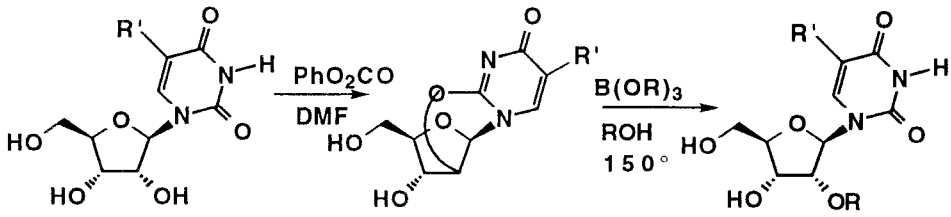
Abstract: A highly efficient, two-step method to make 2'-O-methyluridine is described using only inexpensive reagents and no chromatography. The method is applicable for some other alkyls as well as some other pyrimidine derivatives.

Chimeric oligonucleotides containing 2'-O-alkyl modified ribonucleosides are currently under investigation as candidates for clinical development for antisense and ribozyme applications. As the quantity of required material increases so does the need for more economical methods to prepare the nucleosides. In this report, we focus on an economical synthesis of 2'-O-methyluridine (**5**, see Figure 1) and 2'-O-methyl-5-methyluridine (**6**).

If uridine (**1**) is treated with methyl iodide in the presence of a strong base, methylation first occurs at N-3 and then at either the 2' or 3' hydroxyls with poor selectivity. Most literature methods[1] to make **5** involve multiple blocking/unblocking steps to circumvent the alkylation pattern or difficult chromatographic separations of the isomers.

One way to selectively activate the 2' position is by forming O^2,2'-anhydrouridine (**3**). This material is commercially available in bulk lots or it can be easily prepared in high yield.[2] Treatment of **3** with "soft" nucleophiles such as halogens, thiols or azide affords the desired 2' substituted products in the ribo configuration.[3] On the other hand, "hard" nucleophiles such as methoxide or ammonia attack on the other side of the anhydro linkage resulting in substitution on the 2 position on the heterocycle and leave the hydroxyl on the 2' position in the arabino configuration.[4] It is possible, however, to direct the attack from the desired direction by neighboring group participation of substituents covalently linked to the 3' position[5] as Holy reported[6] with L-3',5'-di-O-benzoyl-O^2,2'-anhydrouridine in the presence of boron trifluoride in methanol to give a mixture of dibenzoylated L-uridines. We wish to report conditions which enable ring opening from the desired direction with oxygen containing nucleophiles without a covalent link.

Using hydrogen fluoride, Fox[3] found it necessary to heat the reaction to 120 °C in a pressure vessel in order to open the anhydro linkage. In a parallel fashion and with methanol as the nucleophile and O^2,2'-anhydro-5-methyluridine (**4**) as the starting material, we first tried boron trifluoride as a Lewis acid catalyst. No reaction was observed until a temperature above 125 °C was reached. The starting material was consumed at 150 °C. **5** was indeed formed but equal amounts of the decomposition products, thymine and arabinosylthymine were also present. Next we tried a milder Lewis acid, trimethyl borate, which would be stable in hot methanol. With this, the reaction went to completion at 150 °C. The solvent and volatile catalyst were removed



1, R' = H
2, R' = CH_3

3, R' = H
4, R' = CH_3

5, R' = H, R = OCH_3
6, R' = CH_3, R = OCH_3
7, R' = H,
R = Phthalimidopropyl

Figure 1

by evaporation and the residue was analyzed by NMR revealing nearly pure **5** with only traces of starting material, thymine and arabinosylthymine.

Optimization of the reaction revealed that at least one equivalent of trimethyl borate was required for completion and more than two equivalents did not improve the reaction. In fact, trimethyl borate must be considered not only a catalyst but a reagent as well and that the attacking methoxide comes from it, probably through coordination of the hydroxyl groups to boron[7] allowing a transient neighboring group participation. Methanol is not required but it is a convenient solvent. Traces of mild base such as sodium bicarbonate accelerate the reaction but too much leads to an increase in side products. The addition of a water scavenger such as trimethyl orthoformate suppresses the formation of arabinosylthymine. The reaction can be done in the temperature range of 120 °C to 200 °C with 150 °C being the optimum. The reaction was successfully scaled up from 1 g in 20 mL to 500 g in a 2 L unstirred pressure reactor. The reaction works equally well with **3**. O^2,2'-Anhydrocytidine hydrochloride works also but the product is contaminated with 30% arabinosylcytosine which is generated during neutralization of the starting material at the start of the reaction.

The use of higher alcohols remains under investigation. In general, the yield drops off as the alcohol size increases or if it contains labile functional groups. One example, using N-(3-hydroxypropyl)phthalimide to give **7**, is included in the experimental section. Commercially unavailable trialkyl borates can easily be generated on a small scale by adding the alcohol slowly to a borane-tetrahydrofuran solution. Larger scale borates can be made by esterification of boric acid in refluxing toluene equipped with a Dean-Stark trap to remove the generated water.

In summary, a new two-step method to make 2'-O-methyluridine has been discovered which requires no chromatography and which consumes starting material, reagents and solvents which total less than $1 per gram of product produced. The reaction works equally well on a gram scale and a kilogram scale and thus enables economical production of material needed for clinical development. The reaction is applicable for some other 2'-O-alkyl pyrimidines giving researchers a new, short, regioselective route to these products.

EXPERIMENTAL

2'-O-Methyluridine (5): In a 2 L stainless steel unstirred pressure reactor were combined O^2,2'-anhydro-uridine (100 g, 0.442 mol), trimethyl borate (100 mL, 0.886 mol),

trimethyl orthoformate (45 mL, 0.442 mol), sodium bicarbonate (150 mg) and methanol (400 mL). The vessel was sealed and placed in an oil bath and heated until an internal temperature of 150 °C was reached which generated 200 psig of pressure. After 42 h the vessel was allowed to cool to room temperature, opened and the reaction solution was transferred to a RB flask and concentrated under reduced pressure. The residue was redissolved in methanol (500 mL) and concentrated again and then dried under vacuum (1 mm Hg) for 1 h to give a foam, 128 g. The foam was dissolved in hot isopropanol (500 mL). The solution was allowed to cool to room temperature and the resulting precipitate was collected by filtration, washed with isopropanol (2x50 mL) and acetone (3x100 mL) and dried (60 °C, 3 h) to 63 g of off-white crystals, mp 157.5-158.5 °C (NMR purity 99+%). The combined filtrate was concentrated to ca 110 mL to give a second crop. The precipitate was collected as before to give another 26 g of product, mp 155-156.5 °C (NMR purity 98%) for a total to this point of 89 g (86%). The filtrate was stripped to a gum/foam (28 g, 11 g over theory) which still contained mostly product contaminated with uracil, boric acid and aliphatic impurities.

2'-O-Methyl-5-methyluridine (**6**): O^2,2'-Anhydro-5-methyluridine (200 g, 0.884 mol), trimethyl borate (200 mL), trimethyl orthoformate (100 mL), sodium bicarbonate (200 mg) in methanol (800 mL) was treated as above. The reaction was cooled, concentrated to a white solid. This was recrystallized from methanol (1.6 L) to give 144 g of first crop, mp 194-195 °C. The filtrate was concentrated (to 300 mL) to give 53 g of second crop, mp 193-194 °C for a total of 197 g (93 %) product (NMR purity 99%). A third crop (9 g) was contaminated with thymine (12%) and an unknown nucleoside (4%).

2'-O-(3-Phthalimidopropyl)-uridine (**7**): N-(3-Hydroxypropyl)phthalimide (19 g, 0.093 mol) was slowly added to a solution of borane in tetrahydrofuran (1 M, 20 mL) with stirring in a 100 mL bomb. Hydrogen gas evolved as the solid dissolved. O^2,2'-anhydrouridine (2.5 g, 0.011 mol) and sodium bicarbonate (5 mg) were added and the bomb was sealed then placed in an oil bath and heated to 175 °C for 72 h. The bomb was cooled to room temperature and opened. The crude solution was concentrated and the residue partitioned between sat'd sodium bicarbonate solution (50 mL) and ethyl acetate (4x50 mL). The combined organic layer was concentrated and the residue was columned on silica gel using ethyl acetate to remove excess phthalimide reagent followed by ethyl acetate-methanol 80/20. As the column fractions were concentrated a precipitate formed which was collected to give 500 mg of pure product as white solid, mp 163-164 °C and 300 mg of less pure residue (18% total yield; yield not optimized).

REFERENCES

1. For examples see a) Inoue, H.; Hayase, H.; Imura, A.; Iwai, S.; Miura, K. Ohtsuka, E. *Nuc.leic Acids Res.*. **1987**, *15*, 6131. b) Wagner, D.; Verheyden, J.; Moffat, J. *J. Org. Chem.* **1974**, *39*, 24. c) Chavis, C.; Dumont, F.; Wightman, R.; Ziegler, J.; Imbach, J.-L.*J. Org. Chem.*. **1982**, *47*, 202.
2. McGee, D.; Vargeese, C.; Zhai, Y.; Kirschenheuter, G.; Settle, A.; Siedem, C.; Pieken, W. *Nucleosides & Nucleotides* **1995**, *14*, 1329.
3. Codington, J.; Doerr, I.; Fox, J. *J. Org. Chem.* **1964**, *29*, 558.
4. Brown, D.; Parihar, D.; Todd, A.; Varadarajan, S. *J. Chem. Soc.* **1958**, 3028.
5. McGee, D.; Sebesta, D.; O'Rourke, S.; Martinez, R., Jung, M.;,Pieken, W. *Tetrahedron Lett.* **1996**, *37*, 1995.
6. Holy, A. *Coll. Czech. Chem. Commun.* **1973**, *38*, 423.
7. We wish to thank Professors Morris J. Robins and Michael Jung for their helpful discussions regarding this reaction and potential mechanisms.